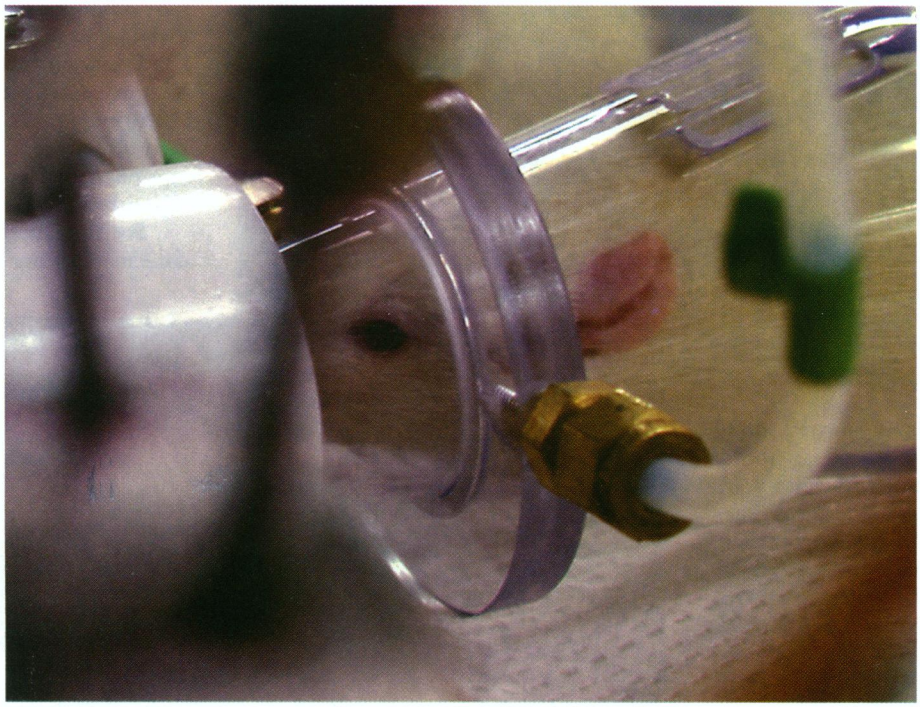
MEASURED BREATH



The mammalian body rids itself of toxic materials in a number of ways: through the intestines in fecal material, through the kidneys in urine, and through the lungs in exhaled breath. Detection of these toxicants may help researchers uncover the means to monitor or screen for other compounds.

Over the years one detection device—the "breathalyzer"—has become familiar because it is used commonly among law enforcement agencies to screen motorists for alcohol on the breath. This device, however, reacts only to alcohol fumes and does not detect the presence of any other volatile organic compound (VOC).

Now the Pacific Northwest National Laboratory (PNNL) in Richland, Washington, in conjunction with the Battelle Memorial Institute in Columbus, Ohio, is developing a device to screen workers for any of over 150 VOCs present in the breath. The Exposure-to-Risk Monitor System (E_2R) can provide breath analysis readings every 1.5 seconds, which is considerably faster than conventional breath analysis systems that provide readings once every 20 minutes or so.

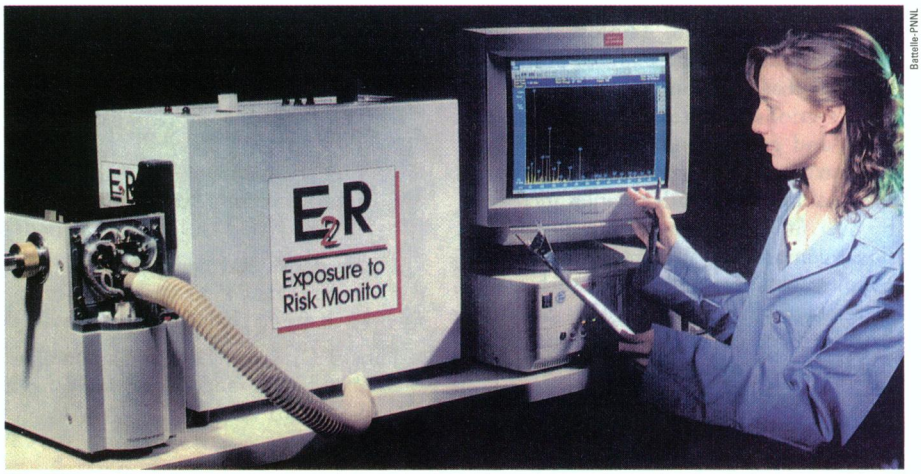
The E_2R system was initiated at Batelle where scientists developed the analytic methods and patented a breath-collection instrument. These were coupled with a mass spectrometer and enabled researchers to measure minute quantities of over 100 volatile chemicals in exhaled breath. Under the leadership of Karla Thrall, project director for the system at PNNL, scientists refined the device further by adding risk assessment algorithms and toxicology models. The models are based on toxicologic studies in animals from which the data are extrapolated to humans. Thus, the E_2R not only measures the level of volatile chemical in the individual's breath, but also can backtrack to determine the dose to which the worker was exposed and can use the toxicology models to calculate potential adverse effects from a given level of exposure.

"There are several advantages to this system," Thrall said. "First, we have a data point every 1.5 seconds for any individual. Second, the readouts are in real time. The person does not breathe into a tank that then must be sent in for laboratory analysis. The analytic process is done on the spot and the reading reflects an accurate measure of the toxins present and the probable dose to which the worker was exposed."

Worker exposure to industrial chemicals often is measured by air monitors or sensors that workers wear. These methods assume that all workers react similarly to the same exposure to a toxic chemical, which in fact is not true. Many chemicals are stored in fat components of the body, so a heavy person may store the chemical and release it slowly over a longer period of time than will a thinner person. The E_2R system measures workers individually and predicts for each person the probability of adverse effects from exposure. In addition, the analysis can be carried out at the site of exposure, in contrast to badge monitors worn by workers, which require laboratory analysis that may take weeks.

"Also, many compounds are not inhaled through the lungs, but are absorbed through the skin," Thrall said. "Until now, there has been no way to track dermal exposure or pick up the presence of toxins absorbed through the skin."

At present the device is portable, though somewhat cumbersome. It is on a wheeled rack and is approximately the size of a five-drawer filing cabinet. One field study has been carried out, and a number of additional studies are planned. "We're available on contract to come into an industrial setting and screen workers," Thrall said. "We hope eventually to make this machine as familiar as the coffee machine to workers in hazardous settings. It will give peace of mind to the worker to be able to know quickly whether his respirator is working, [that] his suit and gloves are not leaking, and his boots are intact. Finding evidence of exposure to hazardous volatile chemicals by short-term screening of workers will provide an opportunity for management to improve safety standards quickly. Workers can be screened at the end of their shift or, for that matter, every half hour or hour if need be," she said.



Battelle-PNNL

Breath catcher. A PNNL scientist checks data generated from a worker's breath test.

Scientists at Battelle have entered the next stage of development for the device. They are looking at the machine's potential as a diagnostic instrument in the medical setting. VOCs often are a byproduct of certain diseases, and the researchers hope to detect them by breath analysis. For example, diabetics often have acetone on the breath, and individuals with cirrhosis of the liver may exhale dimethyl sulfide, both of which can be detected by the monitor. Using the breath monitor would be a quicker means of detecting such biomarkers of disease than the standard laboratory analysis of blood. Much remains to be done in developing the machine for medical application, but the Battelle researchers hope to eventually develop the device in a smaller and less expensive model that can become a standard diagnostic machine in the physician's office.

As to the cost of such a device and of each analytic test, "We don't know that," Thrall explained. "We're developing the technology and intend to commercialize the system with an industrial partner who will market and sell it." When that will occur also has not been decided.

The hint of VOCs. RTI chemists Skip Gaudette (above) and Amy Etheridge (below) conduct breath collection experiments and analyze the components of rats' breath for exposure to volatile organic compounds.

Diagnostic Uses

A similar line of research is being explored by investigators at the Research Triangle Institute and the NIEHS in Research Triangle Park, North Carolina. In these studies, researchers have identified and quantified the hundred or so VOCs that are typically found in the expired breath of rats. Focusing on those breath components that are byproducts of normal metabolic processes, researchers are examining changes in breath components resulting from inhibition or stimulation of certain enzymatic pathways, as well as changes that result from common types of toxic injury.

Animal Research

Initial experiments in the development of a device to measure VOCs have focused on the rat. Investigators have constructed a device to collect expired breath, to the exclusion of nonrespired chemicals, and subject the expired breath to spectrometric analysis.

The rat is placed in a special chamber to restrain its movements, and the rat's head is inserted through a circular port. Purified nitrogen gas is pumped through pores in the port directed backwards toward the rat's body to prevent infiltration of the nose port by ambient air or by VOCs from excreta at the back of the restraining chamber. Such a collar was proved necessary in early models of the system when chemical compounds present in the laboratory air were found in the final analysis. The animal's nose is inserted into a Teflon collar, which prevents absorption of VOCs. The rat breathes air that has been scrubbed of all VOCs during the collection period. The rat's breath is collected over a 90-minute period onto an absorbant, and is then passed through a gas chromatograph and/or a mass spectroscope for analysis. Using this system, breath component analyses were found to be highly reproducible and relatively invariant from day to day, and as the animals aged. There were consistent differences in breath components in male and female rats, and these responded in predictable ways to changes in the sex hormone status of the animals.

Application to Humans

One of the most promising areas of inquiry opened up when researchers inhibited the activities of the cytochrome P450 family

using a relatively nonspecific inhibitor, aminobenzotriazole. The cytochrome P450 enzyme system comprises numerous isoforms that function primarily in oxidative reactions to detoxify drugs, xenobiotics, and endogenous chemicals. When this enzyme family was inhibited in rats, the total mass of breath volatiles increased by over 60%, and several hundred additional components appeared in the breath samples. "The reason this was such a significant finding," said John Bucher, deputy director of the Environmental Toxicology Program at the NIEHS, "is that it suggested that the specific inhibition of one or more cytochrome P450 isoforms might lead to changes in a discrete set of breath components, potentially giving us the ability to identify through a totally noninvasive technique subsets of the population that may be deficient in certain of the isoforms." People deficient in certain P450s have been shown to differ in their ability to detoxify carcinogens or metabolize certain pharmaceuticals at expected rates, leaving them susceptible to drug overdoses. In subsequent unpublished studies, specific breath biomarkers have been identified that reflect the basal activity of cytochrome P450 2E1 in the liver.

A key development in this research has been the development of the Battelle–PNNL E_2R monitoring system. "The difference at present is that PNNL is looking for solvents or other chemicals present in the breath of workers in an industrial setting. We want to look at endogenously generated chemicals in expired breath," Bucher explained. "We are examining patterns of change in all breath components. Now that we know our device can detect minute concentrations of various VOCs, a great deal of information on intermediary metabolism can be amassed, and we believe that different metabolic phenotypes may also be determined in this way."

"This may create a new field of analysis," Bucher continued. "If the changes prove to be consistent from one human subject to the next, it may be possible to apply breath analysis studies in the same way that blood tests now are used in the medical setting. This could be a simpler and more rapid means of identifying metabolic changes so that therapeutic measures could be applied quickly." However, application of the NIEHS system to humans requires much more developmental work and awaits completion of the animal studies.

Larry Blaser

Suggested Reading

Gordon SM, Kenny DV, and Kelly TJ. Continuous real-time breath analysis for the measurement of half-lives of expired volatile organic compounds. J Expos Analysis Environ Epidemiol Suppl 1:41-54 (1992).

Raymer JH, Slauter RW, Velez GR, Gaudette N, and Bucher JR. A nonrebreathing breath collection system for the study of exogenous and endogenous compounds in the Fisher–344 rat. Toxicol Methods 4(4):243–258 (1994).

Mathews JM, Raymer JH, Velez GR, Garner CE, and Bucher JR. The influence of cytochrome P450 enzyme activity on the composition and quantity of volatile organics in expired breath. Biomarkers 1:196–201 (1996).

Thrall KD, Kenny DV. Evaluation of a carbon tetrachloride physiologically based pharmacokinetic model using real-time breath-analysis monitoring of the rat. Inhal Tox 8:251–261 (1996).